Healthy Babies after Intrauterine Transfer of Mosaic Aneuploid Blastocysts

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Chromosomal aneuploidy is recognized as a factor contributing to unsuccessful implantation and spontaneous abortion.

Aneuploidies provide an explanation for the relatively low success rate of in vitro fertilization (IVF) treatments.
IVF – what is the clinical issue?

Embryo aneuploidy and maternal age

- Live birth
- Miscarriage
- Aneuploidy

Age

- <35
- 35-36
- 37-38
- 39-40
- 41-42
- 43-44
- 45>

Percentage
% aneuploidy in embryos increases with maternal age

Data from 7000 blastocysts tested by array-CGH
Transfer of good quality embryos (morphological scoring)
PGS aims to improve embryo selection

PGS is widely used to identify chromosomally normal (euploid) embryos and select them for intrauterine transfer in order to improve the clinical outcome of IVF.
PGS main endpoints

- Improvement of the IVF outcome:
  - increasing the implantation rate, ongoing pregnancy rate and live birth rate for IVF patients
  - reducing the time to pregnancy
  - Selecting the best embryo for single embryo transfer

- Prevention:
  - lowering the incidence of miscarriage
  - reducing the risk of having a baby with an aneuploidy condition
PGS aims to improve IVF outcome

False Positive
False positives decrease cumulative live birth rate
Chromosomal mosaicism

- **Mosaicism**: the presence of chromosomally distinct cell lines within the same embryo

- **mosaic diploid/aneuploid**: mixture of diploid and aneuploid cell lines

- **mosaic aneuploidy**: mixture of cell lines with different chromosomal abnormalities

Chromosomal mosaicism is a relatively common finding in IVF-derived human embryos and may affect:

- **15-90%** of cleavage stage embryos
- **30-40%** of blastocysts

Taylor et al., 2014; Baart et al., 2006; Fragouli et al., 2008; 2011
Trophectoderm biopsy

It is likely that TE samples biopsied from a mosaic blastocyst include more than one cell line.
Aneuploidies in embryos according to the chromosomes involved

Data from 7000 blastocysts tested by array-CGH

Implantation potential

Higher implantation potential

Lower implantation potential

Mosaicism
Common prenatal aneuploidies
Other trisomies (POC)
Double trisomy
Single monosomy
Double monosomy
Complex aneuploidy
Aneuploidies in embryos according to the indication

Data from 7000 blastocysts tested by array-CGH
Aneuploidies in embryos according to maternal age

Data from 7000 blastocysts tested by array-CGH
The clinical impact of mosaicism

- The impact of mosaicism on implantation and the developmental potential of embryos is not known.

- It is reasonable to assume that mosaicism reduces the likelihood of success of IVF.

- Mosaic embryos are not usually transferred because they are deemed abnormal.

- It is important to use CCS methods able to reliably detect chromosomal mosaicism.
Methodologies for 24-chromosome PGS

- Array comparative genomic hybridization (Array-CGH);
- Single-nucleotide polymorphism microarrays (SNP-array);
- Quantitative Polymerase Chain Reaction (qPCR);
- Next-generation sequencing (NGS)

Array-CGH is widely used around the world.

- Extensive validation studies assessing, **Accuracy, Sensitivity, Reproducibility**

- Studies on **mosaicism detection** (Mamas et al. 2012; Novik et al., 2014; Greco et al., in press)

The accuracy to identify chromosomal mosaicism is related to the **limit of detection** of each specific CCS methodology, i.e. the minimum ratio of aneuploid to euploid cells that is needed to detect a chromosomal copy number variation.
NGS advantages

High-throughput capability, with parallel and customizable analysis of multiple embryos (24-96) in a single sequencing run (multiplexing).

Reduced costs per patient.

Possibility to perform combined testing (e.g. Translocation + PGS);

It may also allow simultaneous evaluation of single-gene disorders and abnormalities of the mitochondrial genome, from the same biopsy, without the need for multiple technological platforms.

enhanced detection of partial or segmental aneuploidies, as a result of the potential increase in chromosomal analysis resolution;

Quantification of sequencing read counts provides improved dynamic range and higher sensitivity of quantification, enabling enhanced detection of mosaicism.
aCGH  - 7, +16, -18

NGS
Segmental Imbalance (dup 7p 17Mb)

NGS enables quantification of chromosome copy number
Validation of NGS for PGS

- **preclinical validation study** on single cells to determine the accuracy of the NGS-based 24-aneuploidy screening protocol, demonstrated a high degree of concordance between NGS and aCGH ([Fiorentino et al. Fertil Steril 2014](#)).

- **prospective clinical trial**, involving a double blinded parallel evaluation of embryos at blastocyst stage with both NGS and array-CGH techniques, demonstrated that NGS methodology is reliable, allowing identification and transfer of euploid embryos resulting in ongoing pregnancies ([Fiorentino et al., Hum Reprod 2014](#)).

- Validation of NGS for **mosaicism detection** (unpublished data)
Chromosomal mosaicism can be identified by NGS as low as 20% level

* p<0.05
** p<0.001

A

![Graph A showing trisomy and monosomy copy number vs WGA products percentage](image)

B

![Graph B showing copy number vs aneuploid cells percentage](image)

Fiorentino et al., (unpublished data)
Improved dynamic range of NGS enables enhanced detection and quantification of chromosomal mosaicism
Have mosaic embryos potential for implantation?

Mosaic embryos are not usually transferred because they are deemed abnormal.
Clinical outcome after transfer of mosaic embryos

- 3802 blastocysts analyzed (May-2013 - July-2014)

- 181 (4.8%) diploid/aneuploid mosaic embryos detected

The transfer of mosaic embryos was made available to a consecutive non-selected series of 18 women for whom IVF/PGS had resulted in no euploid embryos

- 8 (44.4%) pregnancies (+² HCG) ensued, of which 6 (33.3%) resulted in the birth of a healthy newborn.

# Mosaic embryos can develop into healthy newborns

## Table 1. Clinical Outcomes of Single Mosaic Blastocysts Transferred.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Chromosomal Constitution</th>
<th>Mosaicism (^\text{‰})</th>
<th>Karyotype</th>
<th>Clinical Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>arr(4)x1, (10)x1</td>
<td>40</td>
<td>46,XX</td>
<td>Baby healthy at birth</td>
</tr>
<tr>
<td>2</td>
<td>arr(6)x1, (15)x1</td>
<td>50</td>
<td>46,XX</td>
<td>Baby healthy at birth</td>
</tr>
<tr>
<td>3</td>
<td>arr(2)x1</td>
<td>40</td>
<td>46,XX</td>
<td>Baby healthy at birth</td>
</tr>
<tr>
<td>4</td>
<td>arr(2)x1</td>
<td>35</td>
<td>46,XY</td>
<td>Baby healthy at birth</td>
</tr>
<tr>
<td>5</td>
<td>arr(5)x1</td>
<td>50</td>
<td>46,XX</td>
<td>Baby healthy at birth</td>
</tr>
<tr>
<td>6</td>
<td>arr(5)x1, (7)x1</td>
<td>40</td>
<td>46,XX</td>
<td>Baby healthy at birth</td>
</tr>
<tr>
<td>7</td>
<td>arr(11)x1, (20)x3, (21)x3</td>
<td>30</td>
<td>NA</td>
<td>No pregnancy</td>
</tr>
<tr>
<td>8</td>
<td>arr(1)x1, (6)x3, (10)x3, (12)x3, (13)x3, (14)x3, (21)x3</td>
<td>50</td>
<td>NA</td>
<td>No pregnancy</td>
</tr>
<tr>
<td>9</td>
<td>arr(3)x1, (10)x3, (21)x3</td>
<td>35</td>
<td>NA</td>
<td>No pregnancy</td>
</tr>
<tr>
<td>10</td>
<td>arr(1)x3</td>
<td>50</td>
<td>NA</td>
<td>Biochemical pregnancy</td>
</tr>
<tr>
<td>11</td>
<td>arr 9p21.2q34.3(26,609,645-140,499,771)x3</td>
<td>45</td>
<td>NA</td>
<td>Biochemical pregnancy</td>
</tr>
<tr>
<td>12</td>
<td>arr(15)x3</td>
<td>30</td>
<td>NA</td>
<td>No pregnancy</td>
</tr>
<tr>
<td>13</td>
<td>arr(18)x1</td>
<td>50</td>
<td>NA</td>
<td>No pregnancy</td>
</tr>
<tr>
<td>14</td>
<td>arr(18)x1</td>
<td>50</td>
<td>NA</td>
<td>No pregnancy</td>
</tr>
<tr>
<td>15</td>
<td>arr(18)x1</td>
<td>40</td>
<td>NA</td>
<td>No pregnancy</td>
</tr>
<tr>
<td>16</td>
<td>arr(4)x1</td>
<td>50</td>
<td>NA</td>
<td>No pregnancy</td>
</tr>
<tr>
<td>17</td>
<td>arr(5)x3</td>
<td>40</td>
<td>NA</td>
<td>No pregnancy</td>
</tr>
<tr>
<td>18</td>
<td>arr 10q21.3q26.3(67,216,644-134,326,648)x3</td>
<td>50</td>
<td>NA</td>
<td>No pregnancy</td>
</tr>
</tbody>
</table>

- **Neg hCG**
- **Biochemical**
- **Healthy at birth**

# Mosaic embryos can develop into healthy newborns

<table>
<thead>
<tr>
<th>Blastocyst N°</th>
<th>Chromosomes</th>
<th>Mosaicism percent</th>
<th>Karyotype</th>
<th>Clinical outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>arr(21)x1</td>
<td>40</td>
<td>46,XX</td>
<td>Baby healthy at birth</td>
</tr>
<tr>
<td>2</td>
<td>arr(1)x1,(13)x3,(15)x3</td>
<td>30</td>
<td>46,XY</td>
<td>Baby healthy at birth</td>
</tr>
<tr>
<td>3</td>
<td>arr(2)x1,(10)x1</td>
<td>50</td>
<td>46,XX</td>
<td>Baby healthy at birth</td>
</tr>
<tr>
<td>4</td>
<td>arr(18)x1</td>
<td>60</td>
<td>46,XY</td>
<td>Baby healthy at birth</td>
</tr>
<tr>
<td>5</td>
<td>arr(17)x3</td>
<td>35</td>
<td>46,XX</td>
<td>Baby healthy at birth</td>
</tr>
<tr>
<td>6</td>
<td>arr(18)x3</td>
<td>50</td>
<td>N/A</td>
<td>bHCG negative</td>
</tr>
<tr>
<td>7</td>
<td>arr(13)x1</td>
<td>50</td>
<td>N/A</td>
<td>bHCG negative</td>
</tr>
<tr>
<td>8</td>
<td>arr(X)x3</td>
<td>40</td>
<td>N/A</td>
<td>bHCG negative</td>
</tr>
<tr>
<td>9</td>
<td>arr(14)x1</td>
<td>60</td>
<td>N/A</td>
<td>bHCG negative</td>
</tr>
<tr>
<td>10</td>
<td>arr(9)x1,(10)x1,(16)x1</td>
<td>50</td>
<td>N/A</td>
<td>Early miscarriage</td>
</tr>
<tr>
<td>11</td>
<td>arr(4)x3,(11)x3,(X)x3</td>
<td>35</td>
<td>N/A</td>
<td>Early miscarriage</td>
</tr>
<tr>
<td>12</td>
<td>arr(3)x1</td>
<td>50</td>
<td>N/A</td>
<td>Biochemical pregnancy</td>
</tr>
<tr>
<td>13</td>
<td>arr(21)x1,(22)x1</td>
<td>30</td>
<td>N/A</td>
<td>bHCG negative</td>
</tr>
<tr>
<td>14</td>
<td>arr(12)x3</td>
<td>50</td>
<td>N/A</td>
<td>bHCG negative</td>
</tr>
<tr>
<td>15</td>
<td>arr(+3)x3,(13)x1,(15)x1</td>
<td>40</td>
<td>N/A</td>
<td>bHCG negative</td>
</tr>
<tr>
<td>16</td>
<td>arr(22)x3</td>
<td>50</td>
<td>N/A</td>
<td>Early miscarriage</td>
</tr>
<tr>
<td>17</td>
<td>arr(3)x3,(7)x3</td>
<td>50</td>
<td>N/A</td>
<td>bHCG negative</td>
</tr>
</tbody>
</table>

Updated data from Greco E, Minasi MG and Fiorentino F. New England Journal of Medicine (in press)
Mosaic embryos can develop into healthy newborns

<table>
<thead>
<tr>
<th>Clinical outcome</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of ET</td>
<td>34</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>35</td>
</tr>
<tr>
<td>No. of (^2) hCG pregnancies</td>
<td>16</td>
</tr>
<tr>
<td>No. of biochemical pregnancies</td>
<td>3</td>
</tr>
<tr>
<td>No. of early miscarriages</td>
<td>3</td>
</tr>
<tr>
<td>No. of ongoing clinical pregnancies per ET</td>
<td>10 (29%)</td>
</tr>
<tr>
<td>No. of pregnancies went to term</td>
<td>10 (29%)</td>
</tr>
<tr>
<td>No. of babies born</td>
<td>11</td>
</tr>
</tbody>
</table>

- No. Of pregnancies went to term
- No. Of biochemical pregnancies
- No. Of early miscarriages
- No. Of negative pregnancies

Updated data from Greco E, Minasi MG and Fiorentino F. New England Journal of Medicine (in press)
Type of aneuploidies detected in mosaic embryos transferred

<table>
<thead>
<tr>
<th>Aneuploidies</th>
<th>N. of blastocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monosomies</td>
<td>1</td>
</tr>
<tr>
<td>Trisomies</td>
<td>7</td>
</tr>
<tr>
<td>&gt;2 aneuploidies</td>
<td>2</td>
</tr>
</tbody>
</table>

- Baby healthy at birth n=11
- No pregnancy n=18
- Biochemical pregnancy n=3
- Early miscarriages n=3

Updated data from Greco E, Minasi MG and Fiorentino F. New England Journal of Medicine (in press)
Mosaicism percentage in transferred embryos

Updated data from Greco E, Minasi MG and Fiorentino F. New England Journal of Medicine (in press)
What about embryos diagnosed as aneuploid by aCGH?

We wondered if the enhanced detection of chromosomal mosaicism by NGS could contribute in identifying mosaic embryos among those diagnosed as aneuploid by aCGH.

We performed a retrospective assessment of WGA products from trophectoderm biopsies, including embryos resulted aneuploid for a single or 2 chromosomes.
12% of the embryos scored aneuploid via aCGH resulted mosaic euploid/aneuploid

<table>
<thead>
<tr>
<th>Samples from aneuploidy Embryos</th>
<th>Array-CGH results</th>
<th>NGS results</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Uniformly aneuploid</td>
<td>129</td>
<td>117 (83%)</td>
</tr>
<tr>
<td>- Mosaic aneuploidy*</td>
<td>0</td>
<td>17 (12%)</td>
</tr>
<tr>
<td>- Uniformly aneuploid/mosaic aneuploidy**</td>
<td>12</td>
<td>7 (5%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of chromosomes assessed</th>
<th>3243</th>
<th>3243</th>
</tr>
</thead>
<tbody>
<tr>
<td>- No. uniformly aneuploid chromosome</td>
<td>252</td>
<td>231</td>
</tr>
<tr>
<td>- No. of anuploid mosaic chromosomes</td>
<td>22</td>
<td>43</td>
</tr>
</tbody>
</table>

*mixture of diploid and aneuploid cell lines, classified as mosaic diploid-aneuploid

**combination of uniformly aneuploid chromosomes and chromosomes with mosaic aneuploidies

Fiorentino et al., (unpublished data)
Improved dynamic range of NGS enables enhanced detection and quantification of chromosomal mosaicism

Fiorentino et al., (unpublished data)
Improved dynamic range of NGS enables enhanced detection and quantification of chromosomal mosaicism.

Fiorentino et al., (unpublished data)
The importance of mosaicism detection

- Importance of the **identification** of mosaic embryos.
  - Correct scoring of the results
  - **avoid discarding** of potentially viable embryos

Mosaic embryos hold the potential to implant, resulting in **chromosomally normal pregnancies** going to term with the birth of healthy babies (Greco et al., NEJM – in press).

Importance of proposing the **transfer** of mosaic embryos to **lower the risk of reducing the chances for IVF patients of achieving a clinical pregnancy**.

This strategy may **important for patients**
  - with **poor ovarian reserve**
  - producing a **limited number of embryos**
  - in which **only chromosomally abnormal embryos** have been detected
**Suggested new method for embryo rating**

<table>
<thead>
<tr>
<th>Euploid</th>
<th>Mosaic</th>
<th>Aneuploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>10%</td>
<td>20%</td>
</tr>
<tr>
<td>30%</td>
<td>40%</td>
<td>50%</td>
</tr>
<tr>
<td>60%</td>
<td>70%</td>
<td>80%</td>
</tr>
<tr>
<td>90%</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

% Chromosomal Mosaicism

Not only black and white, but also different tonalities of gray!
NGS-based PGS has demonstrated to be a robust methodology and a valuable alternative to other currently available CCS techniques.

Quantification of sequencing read counts provides improved dynamic range enabling:

- enhanced detection and quantification of mosaicism
- enhanced discrimination between uniformly aneuploid and mosaic aneuploid embryos

NGS-based PGS has the potential to improve chromosomal diagnosis on embryos.
CONCLUSIONS (II)

Our study shows that mosaic embryos can develop into healthy newborns.

These findings have implications for women who undergo IVF resulting in mosaic embryos but no euploid embryos.

We hypothesize that the extent and type of mosaicism affects IVF success rate.

Our study is small, and additional clinical data must be obtained before this approach can be evaluated for routine integration into IVF-PGS programmes.

Transfer of mosaic embryos with “viable” aneuploidies should be considered with extreme caution.
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